Effect of Food Environment on Staphylococcal Enterotoxin Synthesis: A Review

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ABSTRACT

Effects of various nutritional and environmental factors on growth and enterotoxin synthesis by *Staphylococcus aureus* in model systems and foods are reviewed. Factors discussed include effects of inoculum size, competing microflora, gaseous atmosphere, carbon source, temperature, pH, sodium chloride, water activity, mineral ions and sublethal stress. Areas where additional research is needed are also discussed.

Despite extensive research, *Staphylococcus aureus* remains a major cause of bacterial food poisoning in the United States. During the period 1975-1979, 540 food poisoning outbreaks were reported to the Centers for Disease Control, with *S. aureus* responsible for 28% (153 outbreaks). Among these reported staphylococcal outbreaks, the foods implicated were consumed at home (27%), restaurants (19%), schools (14%) and other known or undetermined localities (40%). Mishandling of foods in foodservice operations appears to be a major cause of outbreaks, followed by mishandling in the home. Few outbreaks appear to be directly attributable to mishandling during food processing operations.

The primary factor contributing to staphylococcal food poisoning outbreaks was improper holding temperatures, with the initial contamination often being traced to poor personal hygiene by food handlers. During 1975-1979, 73% of the staphylococcal outbreaks involved consumption of foods containing meats (red meat, poultry, or fish), with ham being involved in 32% of the reported incidences. [The above information was compiled from Centers for Disease Control Annual summaries (*19-23*).]

The mechanism of staphylococcal food poisoning involves production of an enterotoxin which can elicit the disease response in the absence of viable cells. A number of staphylococcal enterotoxins have been differentiated by serological techniques, and are classified by the letter designations, SEA through SEF (*10,11*). SEC has two different forms, C_1 and C_2, which have different isoelectric points and immunological reactions (*9*). The enterotoxins are composed of single polypeptide chains having a molecular weight of approximately 30,000 daltons (*9*). The various enterotoxins are strain-specific, though it is not unusual to isolate strains capable of synthesizing multiple toxin serotypes. In 200 *S. aureus* strains isolated from foods, Payne and Wood (*81*) found 62.5% to be toxigenic, with SEA-producing strains being the most abundant (47.5%) and SEB-producing isolates being the least common (3.5%). Payne and Wood (*81*) also reported that 21% of the tested strains were capable of producing multiple enterotoxin serotypes. In contrast, Wieneke (*102*) found that almost half (47.7%) of the *S. aureus* strains isolated from raw and cooked foods produced SED; SEC (35.1%) and SEA (26.1%) production was less common. Of 113 strains isolated from food poisoning cases, 77.9% produced SEA, 42.7% produced SED, and 40.7% formed multiple toxin types. The pattern of enterotoxin production was different in *S. aureus* strains isolated from hospital patients (*102*), with SEA, SEB, SEC and multiple toxin production being detected in 44.4, 27.1, 33.3 and 27.9% of the hospital isolates, respectively. Most *S. aureus* strains isolated from processed poultry, poultry processing plants and farms produced SED, while only a small number produced SEA (*39*). Reali (*83*) reported that 82% of the *S. aureus* strains isolated from healthy and infected individuals were able to produce either SEA, SEB, or both; however, the majority (68%) were SEB-producers. Reali (*83*) also examined *S. aureus* isolated from foods not implicated in food poisoning outbreaks, and found that 47% were enterotoxigenic, with SEB-producers again being the predominant toxin type identified. The data of Payne and Wood (*81*), Wieneke (*102*), Harvey et al. (*39*) and Reali (*83*) indicate that no generalization is possible concerning the types of enterotoxin-producing strains that may be isolated from foods or hospital cases.

Staphylococcal enterotoxins are noted for their heat resistance, and typically they cannot be inactivated by normal heat processing of foods, even though the microorganism is readily destroyed. The specific kinetics of enterotoxin inactivation is dependent on heating temperature, pH and heating menstruum (*92*), and presence of pro-

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teinaceous materials protects against thermal inactivation (*60*). Reichert and Fung (*84*) have also shown that upon storage, heat inactivated SEB can undergo re-naturation to its biologically active form.

Staphylococcal enterotoxins have also been implicated as possible suppressors of immunoregulatory mechanisms. Smith and Johnson (*89*) reported that both SEA and SEB inhibited the primary in vitro plaque-forming response of mouse spleen cells against sheep erythrocytes, indicating that the enterotoxins act as immunosuppressants. It has also been reported that SEA is a potent T-lymphocyte mitogen, inducing mitogen-type interferon synthesis in mouse spleen cells (*53*) and human peripheral lymphocytes (*59*). The ability of SEA (and probably the other staphylococcal enterotoxins) to interfere with the functioning of the immune system suggests that there may be a suppression of immune response after a food poisoning episode. This would further suggest that ingestion of *S. aureus* enterotoxins may have health ramifications beyond that of a transitory food poisoning. The immunotoxicology of foodborne toxins has been reviewed by Archer (*3*).

Antibodies against staphylococcal enterotoxins can be detected in the sera of both healthy individuals and those suffering from *S. aureus* infections. Jozefcszyk (*54*) found found that 22.0% of 300 healthy adults were positive for antibodies against staphylococcal enterotoxins (A, B, C_1). Jozefcszyk (*54*) also examined patients with staphylococcal septicemia, respiratory infections, purulent skin infections and wound infections, and found that 49.2% had enterotoxin-positive sera. Anti-SEB and anti-SEA positive sera were found in 35.6 and 15.5% of the infected patients, respectively, while in the healthy individuals, the values were 15.3 and 3.3%. The relationship between the presence of antibodies against staphylococcal enterotoxins and their immunosuppressive activity remains to be investigated.

As previously indicated, the most probable source of *S. aureus* contamination of food is people. A large segment of the population harbors the microorganism as part of the microbiota of the nose, throat and hands, and food handlers can readily contaminate raw ingredients, equipment or finished product *(13)*. Examining nasal swabs from healthy individuals for SEA- and SEB-producing strains of *S. aureus*, Reali (*83*) found that 76% of the isolates were capable of producing one or both of the enterotoxins, with SEB-producing strains being the predominant toxin type identified. Reali (*83*) concluded that *S. aureus* inhabiting the nasal passages is likely to be a major source of *S. aureus* contamination of foods.

Generally, growth of *S. aureus* is necessary for enterotoxin production, though toxin production has been observed in experimental resting cell cultures (*68-70*). However, enterotoxin production does not always accompany growth, particularly in food products. It is not clearly understood why specific food products permit growth but not enterotoxin formation. Identification of key parameters that prevent enterotoxin synthesis in these foods would clearly be useful in formulating other products such that they would be resistant to potential *S. aureus* food poisoning problems. The objective of the present review is to summarize research that has characterized how various parameters of foods affect growth and enterotoxin synthesis by *S. aureus*, and to identify where additional research is needed.

INOCULUM SIZE AND COMPETING MICROBIOTA

Theoretically, a single *S. aureus* cell should be capable of initiating growth and enterotoxin production in food if growth conditions are adequate for the microorganism. However, for *S. aureus* to grow to large populations in a food product, it must be capable of competitively overcoming other microorganisms that may be present. A key environmental determinant is temperature, and staphylococci do not grow in adequately refrigerated foods (*1,36*). In temperature-abused heat-processed foods, particularly those to which salt or some other water activity (a_w) reducing agent has been added, *S. aureus* present as a post processing contaminant will have a competitive edge due to its ability to tolerate lower a_w values as compared to most other microorganisms associated with foods (*74*). However, in temperature-abused raw foods, small numbers of *S. aureus* may not be competitive, and thus inoculum size and intrinsic microbiota become important determinants of a food's inherent resistance to growth and enterotoxin production by *S. aureus*.

Various investigations have demonstrated that experimentally, relatively small inocula of *S. aureus* can lead to growth and concomitant enterotoxin production. Using cooked and raw pork and beef, as well as canned ham, Casman et al. (*18*) found that inoculation with approximately 250 *S. aureus*/cm^2 of meat surface resulted in growth and SEA production at 30°C. In this study, raw meat samples were obtained in a way to minimize competing microorganisms. Genigeorgis et al. (*33,34*) also obtained growth and SEB/SEC production on cooked beef, pork and ham in conjunction with small inocula (10^3/g), depending on the salt, nitrite, pH and temperature levels of the meat. Genoa salami meat mixture inoculated with 10^3, 10^5 or 10^7 *S. aureus*/g supported growth at all inoculum levels; however, SEA production was detected only with the 10^5 and 10^7 inocula (*61*). Inoculating whole milk, skim milk, whipping cream, or half and half with 10^3 staphylococci/ml, Ikram and Luedecke (*50*) found that growth and SEA production occurred in conjunction with a 37°C incubation, but little growth and no enterotoxin were detected at 22°C. Ibrahim et al. (*49*) found that when pasteurized milk inoculated with 5-80 *S. aureus*/ml was used for Cheddar cheese production in conjunction with an inactivated starter, SEA was detectable in cheeses ripened at 11°C. Lee et al. (*63*) reported that inoculating pasta dough with 50-100 staphylococci/g resulted in growth and SEA production at both 25°C and 35°C. Thus, it appears that at least experimentally, fewer than 100 *S. aureus*/g can grow in foods to populations able to produce enterotoxin.

Generally, low levels of *S. aureus* are not competitive in raw foods, and a number of microorganisms associated with foods influence growth of *S. aureus* in associative

culture. When inoculated into media containing a second microbial species, *S. aureus* can be inhibited, stimulated or unaffected by the effector species (*37*). At a ratio of effector organism to *S. aureus* of 100:1, coliforms, *Proteus* spp. and lactic acid bacteria inhibited growth of staphylococci, with the inhibitory effect being more pronounced at 15 than at 30°C (*28,55*). Similarly, *Pseudomonas* and *Archromobacter* species inhibited staphylococcal growth more effectively at 10 than 22°C (*88*).

Haines and Harmon (*38*) demonstrated that when *Streptococcus lactis* (10^5/ml) was grown in associative broth culture (30°C) with *S. aureus* (10^5/ml), the staphylococci increased to approximately 10^6ml, but no enterotoxin production was detected. On the other hand, associative cultures of *S. aureus* and *Pediococcus cerevisiae* allowed *S. aureus* to increase from 10^5 to 10^8/ml. However, the presence of *P. cerevisiae* did result in an approximate 20-fold decrease in levels of SEA, SEB and SEC produced, and no SED production was detected. Using associative cultures of *S. aureus* and *Pseudomonas aeruginosa*, Collins-Thompson et al. (*25*) found that there was a marked decrease in SEB synthesis. The staphylococci only grew for a short period, and they lost their tolerance to 7.5% NaCl. This loss of salt tolerance suggests that the cells had incurred membrane damage or injury, presumably due to the production of staphylolytic enzymes by the pseudomonad. Bluhm and Ordal (*12*) have shown that injured *S. aureus* (as determined by loss of salt tolerance) have severely limited catabolic and anabolic activity.

When partially purified SEA was added to microbiological media inoculated with various microorganisms, Chordash and Potter (*24*) found that *Bacillus, Pseudomonas, Escherichia, Candida,* and *Saccharomyces* species had no effect on recoverable toxin levels. However, species of *Lactobacillus, Streptococcus,* and *Leuconostoc* decreased SEA levels. This apparent destruction of SEA was not related to the decreased pH associated with lactic acid cultures since uninoculated SEA-containing media acidified with lactic acid to pH 3 to 6 had no effect on recoverable enterotoxin.

McCoy and Farber (*65*) reported that when a variety of common food bacteria (both gram positive and gram negative) were added singly to beef or ham slurries, *S. aureus* growth was inhibited, or SEA production was decreased with little or no effect on growth. McCoy and Farber (*65*) also reported that *S. aureus* grown in the presence of *Bacillus cereus* resulted in increased SEA production. In low count (10^4/ml aerobic count) raw milk and pasteurized milk, Donnelly et al. (*29*) found that *S. aureus* grew and produced SEA at 20, 25 and 30°C, but not at 10°C. In high count (5×10^6/ml) raw milk, SEA production was only detected in conjunction with a 35°C incubation. Tatini et al. (*94*) reported that SEA production was not detected in blue cheese manufactured from milk inoculated with *S. aureus*, even though bacteriophage inactivation of the starter culture allowed staphylococci to reach 5×10^7/g. Tatini et al. (*94*) attributed the lack of SEA synthesis to the presence of the microbiota of the raw milk; however, the possibility that *Penicillium roqueforti* was inhibiting enterotoxin biosynthesis was not investigated. Using culture media, milk and ham, Noleto and Bergdoll (*79*) found that when enterotoxigenic *S. aureus* was grown in conjunction with nontoxigenic strains, SEA, SEB, and SED production was evident, even though the nontoxigenic strains were present in large excess.

Additional research is needed to more fully determine the effect of competing microorganisms on production or stability of staphylococcal enterotoxins, particularly in low-acid foods. In high acid products where acidification is achieved by fermentation by lactic acid bacteria, additional research should be directed for optimizing their inhibition of *S. aureus*, thereby more fully assuring the safety of these products. Furthermore, lacic acid starter cultures are reported to produce antibiotic-like substances (*4*), and it is possible that this characteristic could be used to inhibit staphylococcal growth and/or enterotoxin formation even in low acid products.

ATMOSPHERIC COMPOSITION

Various atmospheric compositions have been reported to affect growth and enterotoxin production by *S. aureus;* however, the results have often been contradictory, and definitive studies are lacking. Metabolically, *S. aureus* is classified as a facultative anaerobe that grows more rapidly and abundantly under aerobic conditions (*14,41*). Therefore, aeration would be expected to have a positive effect on growth and subsequent entertoxin formation. McLean et al. (*66*) and Dietrich et al. (*27*) found that aeration by shaking at 37°C allowed *S. aureus* incubated in air to produce approximately 10-fold more SEB as compared to similar cultures incubated in an atmosphere of 95% N_2 + 5% CO_2. Use of aerated conditions (shaken flasks) also appears to increase the yield of the other enterotoxins. For example, Woodburn et al. (*104*) found that shaken incubation greatly increased SEA, SEB and SEC production as compared to static incubation.

Dissolved oxygen (DO) levels appear to be more influential in controlling growth and enterotoxin formation than the actual rate of aeration or agitation. At 100% DO, growth of *S. aureus* at 37°C was maximal, but there was no synthesis of SEB (*16*). Decreasing the DO to 50% decreased growth (as measured by Klett meter), but enterotoxin production increased markedly. Maximal SEB production occurred in conjunction with a DO of 10%. In contrast to SEB, synthesis of SEA appears to be more directly related to growth of *S. aureus* and less influenced by environmental conditions. Carpenter and Silverman (*17*) did not observe an optimal DO for SEA production, and concluded that SEA synthesis is independent of this parameter. The data obtained with culture media indicated that abundant toxin production was not obtained in the absence of aeration, though small amounts of enterotoxin were found in conjunction with low oxygen tensions.

In foods, *S. aureus* growth and enterotoxin formation have been observed under anaerobic conditions; however, like culture media, enterotoxin yields are greater under aerobic conditions. Slices of Canadian bacon inoculated

with *S. aureus* and stored at 37°C in air, N_2, vacuum (flushed with N_2), and 5% CO_2 + 95% O_2 supported enterotoxin formation regardless of atmospheric composition (*95*). However, more toxin was produced in those atmospheres containing oxygen. Genigeorgis et al. (*33*) reported that SEB was produced in hams incubated at 22 and 30°C under both aerobic and anaerobic conditions, with growth and toxin production occurring more rapidly under aerobic conditions. It was also observed that even when hams were held under ideal conditions for *S. aureus* (39°C, pH 5.3, NaCl in brine 9.2%), SEB was not detected in all samples (regardless of atmosphere), even though the *S. aureus* count was high. While growth of *S. aureus* in hams may be anticipated, actual production of enterotoxin in any particular sample could not be predicted (*33*). Since ham is a major source of staphylococcal food poisoning, the exact parameters leading to enterotoxin production in this product should be more fully elucidated.

Prawns inoculated with *S. aureus* and incubated in air at <26°C had detectable SEB production after 7 d (*5*). However, no SEB was detected when prawns were stored in an atmosphere of 95% N_2 + 5% CO_2. Using a model sausage system (beaker sausage), Barber and Deibel (*6*) demonstrated that *S. aureus* could produce SEA at 37°C within 24 h in atmospheres containing 10, 15 and 20% O_2. At 5% O_2, toxin was detected after 48, but not 24 h. In a 100% helium atmosphere, SEA was not detected within 120 h, even though *S. aureus* was at a level capable of supporting toxin production (5×10^7/g). Lee et al. (*61*) studied staphylococcal growth and enterotoxin production in Genoa salami. At initial *S. aureus* levels of 10^5 or 10^7/g (but not 10^3), SEA was detected on the surface of the salamis (outer 10 mm of 90-mm sausage), but not in the core (inner 70 mm). *S. aureus* counts were always higher at the surface, reflecting the limited O_2 tension in the interior of the product. It has also been shown that nitrite more effectively inhibits *S. aureus* under anaerobic conditions (*14,90*).

Bennett and Amos (*7*) examined the effect of N_2 storage on enterotoxin formation in sausage, turkey and hamburger sandwiches that had been inoculated with enterotoxigenic *S. aureus* at a level of 30 cells/g. No toxin formation was detected after 31 d when the sandwiches were incubated at 8 or 12°C. Enterotoxin production (SEA, SEB, SEC, SED, SEE) did occur in N_2-packed sausage and hamburger sandwiches incubated at 26°C; however, even at this temperature, the turkey sandwiches did not support enough growth to result in detectable levels. Bennett and Amos (*7*) also found that sandwiches containing detectable levels of enterotoxin were organoleptically acceptable, and concluded that proper refrigeration of these products is needed to avoid potential food poisoning outbreaks.

It appears that alteration of atmospheric composition can influence the potential for enterotoxin production, particularly when oxygen is eliminated or reduced. Additional research is needed to further characterize this effect and thus better use this parameter for controlling staphylococcal food poisoning. Further work is also needed to determine how other atmospheric compositions affect the microorganism. It is expected that use of modified atmospheres (containing CO_2 levels ≥20%) for preservation of fish and meat products will increase in the near future (*29a,103*). Therefore, determination of how CO_2 levels greater than 5% affect *S. aureus* growth and enterotoxin production may be a worthwhile avenue for further research.

CARBON SOURCES

Addition of readily metabolizable carbon sources such as glucose or pyruvate to casein hydrolysate medium represses synthesis of SEB (*76,78*). Similarly, addition of glucose or glycerol to a chemically-defined medium resulted in a marked decrease in biosynthesis of SEA, SEB and SEC (*52*). Metabolism of glucose by *S. aureus* resulted in a marked decrease in pH (<5.0) due to the incomplete oxidation of glucose, but Morse et al. (*78*) suggested that the repression of SEB synthesis was not due to the altered pH. In contrast, Metzger et al. (*72*) suggested that pH rather than catabolism of glucose was the key factor involved in glucose-associated inhibition of SEB synthesis.

Conditions favoring the oxidative decarboxylation of pyruvate favored repression of SEB synthesis (*76*). Elimination of thiamine from the medium prevented this metabolic reaction, and also prevented repression of enterotoxin synthesis by glucose or pyruvate. Morse and Mah (*77*) found that glucose repression of SEB synthesis could be reversed by abruptly shifting aerobic cultures to anaerobiosis (95% N_2 + 5% CO_2). This derepression of toxin formation could be prevented by addition of nitrate to the cultures that had been shifted to anaerobic conditions. Under aerobic conditions, nitrate did not potentiate the repressive effect of glucose. Presumably the effect of nitrate relates to its ability to replace oxygen as a terminal electron acceptor.

Employing agitated cultures, Jarvis et al. (*52*) found that glucose or glycerol severely repressed SEA, SEB and SEC production by *S. aureus* grown in a chemically defined medium. When a fermenter was used to maintain the pH at 6.5 and keep the level of glucose or glycerol constant, repression of enterotoxin synthesis still occurred, though to a lesser degree. These results suggest that repression of enterotoxin production can only be partially attributed to decreased pH.

Iandolo and Shafer (*48*) reported that addition of 2-deoxyglucose (2-DOG), a nonmetabolizable glucose analog, inhibited both growth and SEB synthesis. Unlike glucose, inhibition by a 2-DOG was permanent. Simultaneous addition of glucose and 2-DOG led to a partial reversal of the repressive effect of 2-DOG on SEB synthesis. Addition of cyclic 3', 5'-adenosine monophosphate (cAMP) did not relieve the repressive effects of either glucose or 2-DOG. Iandolo and Shafer (*48*) also studied the effect of the nonmetabolizable glucose analog, alpha-methylglucoside (AMG), on SEB synthesis by *S. aureus*. Surprisingly, AMG had a slight stimulatory effect on SEB production. While glucose does suppress SEB synthesis, it does not appear to act in a manner similar to the catabolite repression of β-galactosidase in *Escherichia coli* (*82*). This supposition is based on the observations that (*a*) 2-DOG

and AMG produce a differential response, and (*b*) suppression of SEB production was not reversed by addition of cAMP (*48*).

Keller et al. (*58*) studied the effect of amino acids as energy sources for growth and SEB production by *S. aureus*. Glutamate, proline, histidine, aspartate, alanine, threonine, serine or glycine acted as energy sources for the microorganism when they were added individually to a salts-vitamin-amino acid medium. When the concentration of the amino acid serving as an energy source was increased from 0 to 10 m*M*, there was an increase in cell dry weight by 1.1- to 2.1-fold for all of the amino acids except glutamate which produced a 4-fold increase. Increasing proline, histidine, alanine, or serine levels from 0 to 10 m*M* produced only a slight increase in SEB synthesis, while elevating the concentrations of aspartate, glycine, threonine or glutamate depressed SEB production by 25, 54, 58 and 88%, respectively (*58*). Inhibition of SEB synthesis by amino acids does not appear to be similar to that associated with the use of glucose as an energy source.

It does not appear as if investigations have been carried out to determine if sugars or other energy sources could be used to suppress toxin formation in foods. Studies examining the effect of glucose and other energy sources that repress toxin formation have been of short duration (<20 h), and have been limited to using microbiological media. While at present, carbon source suppression of enterotoxin synthesis is only a laboratory phenomenon, it may have applicability for controlling *S. aureus* enterotoxigenesis in food, and warrants study in food systems.

TEMPERATURE

Tatini (*91*) summarized the cardinal temperatures for *S. aureus* growth as being a range of 7 to 47.8°C, and an optimum of 37°C. The corresponding values for enterotoxin production were a range of 10 to 46°C, and an optimum of 40 to 45°C. Using brain Heart Infusion (BHI) broth, McLean et al. (*66*) found that the amount of growth obtained after 112 h with incubation temperatures of 16, 20 or 37°C was similar, but the amount of SEB synthesized decreased markedly with decreasing temperature. The level of SEB production detected was 8, 20, and 340 μg/ml at 16, 20 and 37°C, respectively. Similarly, Hojvat and Jackson (*40*) reported that SEB production in agitated BHI cultures was 1000 μg/ml after 48 h at 35°C, while incubation for up to 8 d at 30, 25 and 20°C resulted in toxin levels of 300, 150, and 25 μg/ml, respectively. No enterotoxin was detected within 8 d in cultures incubated at 15°C. Also employing BHI, Scheusner et al. (*87*) reported that temperature ranges for growth and toxin production by SEA, SEB, SEC, and SED-producing strains were similar, though not identical.

Using casein hydrolysate medium, Dietrich et al. (*27*) found that temperatures of 25 to 40°C supported approximately the same amount of growth after 25 h, while decreasing the temperature to 20°C, resulted in a 50% decrease in growth. SEB production was greatest at 37°c, and and a 2°C differential (i.e., 35°C) resulted in an approximate 50% reduction in enterotoxin production. Incubation temperatures of 20 and 40°C reduced SEB production by 99 and 60%, respectively, and no growth or toxin production was evident at 45°C. However, Vandenbosch et al. (*101*) reported that the optimum temperature for SEB and SEC synthesis in casein hydrolysate medium was 40°C, with no toxin being detected at either 10 or 50°C. Hughes and Hurst (*43*) found that the optimum temperature for SEA and SEB production in broth cultures was 43 and 39°C, respectively.

Results from studies employing microbiological media as model systems suggest that (*a*) the optimum temperature for enterotoxin production is a few degrees higher than that for growth, and (*b*) temperature changes affect enterotoxin synthesis more strongly than growth. Results from model system studies appear to be in fair agreement with those obtained from investigations employing actual food samples.

The temperature range supporting *S. aureus* growth in a variety of foods (sterile custard, ham salad and chicken a la king) was 6.7 to 45.6°C (*2*), with no growth being detected at temperatures <5.6°C. Donnelly et al. (*29*) found that pasteurized milk inoculated with *S. aureus* (10^4/ml) had detectable levels of SEA in 12 h when incubated at 35°C, while toxin was detectable after 18, 24 and 48 h when incubated at 30, 25 and 20°C, respectively. Neither growth nor toxin production were observed after 168 h at 10°C. Donnelly et al. (*29*) also demonstrated that with a higher initial inoculum level (10^6/ml), SEA was detected sooner at all incubation temperatures except 10°C, where growth and toxin production were again not detected. Scheusner and Harmon (*86*) found that vanilla pudding inoculated with *S. aureus* (10^5/g) supported SEA, SEB, SEC and SED production over a range of 10 to 45°C.

Genigeorgis et al. (*33*) found that hams stored anaerobically at 10, 22 and 30°C supported growth and SEB production. Interestingly, many ham samples contained no detectable levels of enterotoxin even though *S. aureus* had attained populations normally associated with toxin synthesis. As previously suggested, it appears that there may be one or more unidentified factors in hams (and probably other foods) that influence enterotoxin production without affecting growth. Identification of these factors might lead to development of improved methods for controlling staphylococcal food poisoning.

Foods subjected to temperature abuse (>10°C) must be considered potential candidates for staphylococcal enterotoxin production, with the ensuing possibility for a food poisoning outbreak. The literature suggests that the amount of enterotoxin synthesized by *S. aureus* decreased dramatically when the microorganism is grown at 20 to 25°C, even though final cell densities are similar over a wide range of temperatures. However, even at the lower temperature ranges, an extended temperature abuse period could allow sufficient toxin synthesis to pose a food poisoning risk. Bergdoll (*8*) concluded that less than 1 μg of enterotoxin/100 g of food will produce food poisoning symptoms in sensitive individuals. Whether the reduction in enterotoxin production at lower temperatures is due to a direct effect on toxin biosynthesis or the result of some

change in the physiological or nutritional status of the microorganism remains to be elucidated.

ACIDITY

Tatini (*91*) indicated that the optimum pH for *S. aureus* growth was between 6.0 and 7.0, but the microorganism could grow over a pH range of 4.0 to 9.8. As a general "rule of thumb," when other cultural parameters become non-optimal, the pH range tolerated by *S. aureus* is reduced. For example, Genigeorgis (*31*) found that the lowest pH that permitted growth and enterotoxin formation by aerobically cultured cells was 4.0, while the lowest pH values that supported growth and enterotoxin production in anaerobic cultures were 4.6 and 5.3, respectively.

In casein hydrolysate medium, Kato et al. (*56*) found that initial pH values of 5.0 to 8.0 yielded similar amounts of SEA production. Using a variety of media, Reiser and Weiss (*85*) also found that production of SEA was independent of initial pH when it was within the range 5.3 to 6.8. Production of SEB was influenced to a greater extent by initial pH as compared to SEA. Using BHI, Genigeorgis and Sadler (*32*) found that SEB production was similar over an initial pH range of 6.0 to 6.9, but decreasing the pH to 5.5 resulted in a 4-fold decrease in toxin formation. No SEB production was detectable in conjunction with an initial pH of 5.1. Scheusner et al. (*87*) found that SEB could be detected in 4 to 6 h in BHI cultures (37°C) adjusted to pH 7.14 to 7.95, while no toxin synthesis was detected in conjunction with initial pH values of 5.02 or 9.08. Reiser and Weiss (*85*) found that an initial pH of 6.8 gave higher yields of SEB and SEC than initial pH values of 5.3 to 6.0.

Barber and Deibel (*6*) found that the lowest pH values that supported SEA, SEB, SEC_2 and SEE synthesis in buffered BHI medium incubated aerobically were 4.9, 5.0, 4.9 and 4.8, respectively. Anaerobically, the minimum pH for SEA, SEB and SEC_2 production was 5.7, while the minimum for SEE was 6.0. However, strain differences were observed, with the minimum pH for SEA production ranging from 4.9 to 5.7 aerobically, and 5.7 to 7.0 anaerobically.

The pH optimum for SEA synthesis by nonreplicating cells of *S. aureus* in either the presence or absence of a nitrogen source was between 6.6 to 7.0 (*70*). The optimum pH for SEB production by nonreplicating cells was between 8.0 to 8.5 in the absence of a nitrogen source, but 7.0 to 7.5 when a nitrogen source was included (*69*).

Using a fermenter containing casein hydrolysate medium, Metzger et al. (*72*) and Carpenter and Silverman (*16,17*) found that the highest concentrations of both SEA and SEB were produced when the pH was maintained at 7.0. Holding the pH constant at 6.0 or 8.0 led to drastic decreases in the amount of SEB produced (*72*). Jarvis et al. (*51*) found that 3 of 5 SEA-producing *S. aureus* strains produced more toxin when the pH was controlled at 6.5 (using a fermenter as compared to agitated flask cultures where the pH was uncontrolled). The elevated production of staphylococcal enterotoxins in pH-controlled environments may have significance in terms of food poisoning outbreaks, since many foods are strongly buffered at pH values between 6.0 and 6.5. Experimental determinations of SEA-producing capacity of *S. aureus* strains may give a false picture because these strains may produce little or no toxin under conditions normally employed for culturing the microorganism in the laboratory, but could produce significant amounts of SEA in highly buffered foods.

Using SEA-, SEB-, SEC- and SED-producing strains of *S. aureus*, Scheusner and Harmon (*86*) showed that enterotoxin was produced in a variety of foods having pH values ranging from 5.5 to 6.6, but no enterotoxin was detected in foods having a pH <5.0. Genigeorgis et al. (*33*) reported that the minimum pH for anaerobic production of SEB in ham slices was 5.3 at incubation temperatures of 22 and 30°C, but 5.6 at 10°C.

The acidulant used to adjust the pH of a food may also be a factor affecting enterotoxin synthesis. For example, Tatini et al. (*93*) found that when milk was adjusted to pH 4.5, 5.0, 6.0 or 6.4 with HCl, SEA was produced at all pH levels. However, when the milk was adjusted with lactic acid, growth and toxin formation did not occur at pH 4.5, though toxin production was evident at the higher pH levels.

High *S. aureus* counts and SEB production were found in Swiss cheeses having pH values of 5.4 to 5.7 (*96*). Swiss cheese normally has a pH of 5.6 to 5.7, so insufficient acid production does not appear to explain the presence of enterotoxin in the cheeses. A large competing inoculum of *S. aureus* in the milk, rather than starter culture failure, was the most probable reason for the SEB in the cheeses. Todd et al. (*96*) concluded that in addition to having an active starter culture, the level of *S. aureus* must be minimized. Zehren and Zehren (*105*) found that the level of titratable acidity was a better indicator than pH for determining if Cheddar cheeses contaminated with *S. aureus* would support SEA production.

Only a limited amount of research has been done on the relationship between pH and enterotoxin synthesis in foods. Foods having pH values below 5.0 do not appear to support enterotoxin synthesis. Thus fermented foods or acidified foods (acetic or lactic acids) that have been properly prepared should not support enough *S. aureus* growth to lead to enterotoxin biosynthesis, even if temperature-abused (*73*). With fermented foods, a key determinant is how quickly an inhibitory pH is reached. However, it is important to note that many foods have pH values above 5.5, and if these foods are temperature-abused, enterotoxin production may result.

SODIUM CHLORIDE

Tatini (*91*) reported that the optimum NaCl level for *S. aureus* growth was 0%, with a range of 0 to 20%. Enterotoxin production was also optimal with 0% NaCl, but occurred over a range of 0% to 10%. It should be noted that *S. aureus*, like other microbial species, requires the presence of various minerals, and presumably may require trace amounts of both Na^+ and Cl^-. Over a sodium

chloride range of 0 to 10%, Markus and Silverman (*70*) found that the amount of SEA produced per unit of cell mass remained constant. Thus, as the NaCl increased, both cell growth and SEA production decreased proportionally. Using a SEA-producing strain of *S. aureus*, Hughes and Hurst (*43*) found that the addition of 1 *M* NaCl (5.8%) increased the microorganism's upper temperature limit for growth from 45.5 to 47.0°C. Similarly, the upper limit for SEA production was raised from 45.5 to 46.5°C. In this instance, the salt may function to stabilize various enzymes so that they were not denatured by the heat. Similar effects were seen with a SEB-producing strain of *S. aureus*. Hurst et al. (*46*) demonstrated that other salts (KCl, NH_4Cl, and $MgCl_2$) were also effective at raising the temperature limit for growth of a SEB-producing strain of *S. aureus*.

McLean et al. (*66*) demonstrated that the final population density of *S. aureus* cultures (as measured by Klett meter) decreased by 20% when NaCl levels were increased from 0% to 10%. However, SEB synthesis decreased from approximately 500 μg/ml to 0 μg/ml with increasing NaCl levels. It appears that unlke SEA formation, SEB production is more strongly inhibited by NaCl levels than is growth of the microorganism.

Using BHI having initial pH values of 6.0 to 6.9, Genigeorgis and Sadler (*32*) found abundant SEB production after 48 h at 37°C in the presence of NaCl levels of 2 to 6%. SEB production was reduced with 8% NaCl, and no toxin was detected in conjunction with 10% NaCl. When the initial pH was adjusted to 5.5, progressively less SEB was produced as the NaCl level was raised from 2 to 6%, and no toxin was detected in conjunction with 8 and 10% NaCl. At pH 5.1, no detectable SEB production was observed with NaCl concentrations >2%. It appears that there is an interaction between initial pH and NaCl concentration that affects SEB synthesis.

Hojvat and Jackson (*40*) studied the effect of temperature and NaCl level on growth and SEB synthesis by *S. aureus* in BHI. The microorganism did not grow at 4°C over an 8-d period, regardless of NaCl concentration. At 15°C, growth occurred in conjunction with 0, 4 and 8% NaCl, but not at 12%. At 20 and 35°C, growth occurred at all NaCl levels tested (0 to 12%). In the absence of NaCl, SEB was produced at 20 and 35°C, but not at the lower incubation temperatures. At 4 and 8% NaCl, SEB was detected at 35°C only, and no toxin was detected in conjunction with 12% NaCl, regardless of incubation temperature. However, Baird-Parker(*5*) criticized the results of Hojvat and Jackson (*40*) because of the low sensitivity of the SEB assay employed. Using ham slices, Genigerogis et al. (*33*) found that SEB was produced anaerobically at NaCl levels ranging from 1.4 to 6.4%; however, many hams that fell within that range of NaCl concentrations did not contain detectable SEB levels, even though *S. aureus* growth was such that enterotoxin synthesis could be anticipated. The lack of SEB production did not appear to be related to pH, nitrite levels or incubtion temperature of the ham slices.

Using large inocula (10^8/ml) of *S. aureus*, Genigeorgis et al. (*35*) demonstrated that casein hydrolysate medium containing 0, 4 and 8% NaCl, and adjusted to initial pH values from 4.5 to 8.5, supported SEC production. When the NaCl concentration was increased to 10%, the pH range that supported SEC production was reduced to 5.5 to 7.3. No enterotoxin production was evident at 12% NaCl, regardless of initial pH.

Growth of SEB-producing *S. aureus* appears to be less affected by NaCl levels than is toxin production (*35,40,66*). On the other hand, growth and enterotoxin production seem to be equally affected in SEA-producing strains (*70*). Thus the response of *S. aureus* to NaCl levels appears to be dependent on the serotype of the enterotoxin produced. Neither growth or SEA (*70*) and SEB (*66*) production appear to be affected by the presence of 200 ppm nitrite or 1000 ppm nitrate. Other compounds used in processed meats to not appear to have been studied for their effects on staphylococcal growth and enterotoxin synthesis.

Recently, in an attempt to reduce the consumption of sodium, it has been suggested that the sodium chloride added to foods be replaced partially or completely by KCl, $CaCl_2$, $MgCl_2$, or a combination of salts (*80*). However, the effect of these salts on staphylococcal growth and enterotoxin formation is not known. In one of the few studies on this topic, Morita et al. (*75*) demonstrated that addition of Mg^{++} to growth media stimulated production of enterotoxin by *S. aureus*. It appears prudent to recommend that before any extensive substitution of NaCl by other salts is made, the effect of these NaCl-replacements on *S. aureus* (and other foodborne pathogens) be evaluated.

WATER ACTIVITY

Probably more than any other food poisoning bacteria, *S. aureus* has been examined extensively for its ability to tolerate decreased water activities (a_w). This interest is largely due to the microorganism's ability to grow over a much wider a_w range than other food-associated pathogens. The optimum a_w for *S. aureus* growth is >0.99, with a range of 0.83->0.99 (*91*). The requirement for enterotoxin formation is similar, with an a_w range of 0.86->.99, and an optimum of >0.99.

Using NaCl as a humectant, Troller and Stinson (*100*) found that decreasing the a_w of casein hydrolysate medium from 0.996 to 0.91 had only a slight effect on growth of a SEA-producing strain of *S. aureus*. However, cell protein levels were decreased 2-fold, and SEA production was reduced 5-fold. Similar experimentation with a SEB-producing strain again indicated little effect on growth, but cell protein and SEB levels were decreased 2-fold and 43-fold, respectively. Troller and Stinson (*100*) concluded that SEB production is more sensitive than SEA to a_w modification.

The amount that the a_w of a system can be reduced and still support staphylococcal growth is generally greatest when the microorganism's other environmental and nutritional parameters are optimized. When these parameters deviate from their optima, the minimum a_w tolerated by *S.*

aureus is elevated. Some of the parameters identified as influencing the minimal a_w requirements of *S. aureus* include atmospheric composition, temperature, humectant employed and initial pH. Genigeorgis (*31*) reported that the minimal a_w for staphylococcal growth under aerobic conditions was between 0.83 to 0.86, but with anaerobic conditions the minimal a_w was 0.90. Using a mixture of NaCl, KCl and Na_2SO_4 to adjust the a_w of a broth, Lotter and Leistner (*64*) determined that the minimum a_w for both growth and SEA synthesis of two strains of *S. aureus* cultured at 30°C was between 0.864 to 0.867. At 25°C, the minimal a_w increased to between 0.870 and 0.887. Troller (*97*) reported that minimum a_w for SEB production was 0.98 to 0.99 when glycerol was used to adjust a_w. However, when NaCl was used, the minimum a_w was 0.90 to 0.92, and a mixture of NaCl, KCl and Na_2SO_4 permitted enterotoxin production at an a_w <0.90 (*98*). Troller (*98*) reported that when the pH of a broth system was decreased from 6.8 to 5.6, the minimal a_w for SEB production was increased from 0.92 to 0.94, using either NaCl or a salt mixture as the humectant.

Tatini (*91*) reported that *S. aureus* grew in cured beef slurry adjusted to an a_w of 0.86, but not 0.82. SEA was produced at an a_w of 0.88, but not 0.86. *S. aureus* was able to better tolerate reduced a_w in cured pork slurry, with the minimal a_w for growth and SEA production being 0.83 and 0.86, respectively (*91*).

Lee et al. (*62*) studied growth and SEA production by *S. aureus* in precooked bacon. Various a_w levels were attained by altering the duration of the precooking cycle. With bacon stored at 37°C, the minimum a_w values for staphylococcal growth were 0.84 and 0.90 for aerobic and anaerobic (vacuum canned) storage, respectively. In general, as the temperature of storage decreased, the limiting a_w increased. For example, the minimum a_w values for the aerobic and anaerobic storage of bacon at 20°C were 0.88 and 0.91, respectively. Lee et al. (*62*) found that the minimum a_w values for SEA production in bacon stored aerobically and anaerobically at 37°C were 0.84 and 0.90, while at 20°C, the values were 0.88 and 0.94; as the a_w of bacon decreased, the amount of SEA produced was decreased. At comparable a_w values, more SEA was produced at 37°C than 20°C. Formation of enterotoxin in precooked bacon is of concern because it is unlikely that additional cooking of the product would result in a significant inactivation of the heat-stable toxin.

Using glycerol to adjust shrimp slurry to various water activity levels, Troller and Stinson (*99*) found that growth of an SEA-producing strain of *S. aureus* occurred at an a_w of 0.93, but not 0.89. SEA was synthesized at an a_w of 0.95, but not 0.93. The amount of SEA produced in the shrimp slurry ranged from 0.06 to 0.04 $\mu g/10^8$ cells when the a_w was reduced from 0.99 to 0.95. Troller and Stinson (*99*) also reported that in potato dough adjusted with glycerol, staphylococcal growth and SEA production occurred at 37°C in conjunction with an a_w of 0.93, but at an a_w of 0.88, growth or enterotoxin were not detected. Troller and Stinson (*99*) also did similar experimentation employing a SEB-producing strain of *S. aureus*. In shrimp slurry incubated at 37°C, growth occurred at an a_w of 0.91, but not 0.89, and SEB production was detected at an a_w of 0.93, but not 0.91. The amount of SEB detected was 1.57, 0.80, 0.83 and 0.75 $\mu g/10^8$ cells for a_w levels of 0.99, 0.97, 0.95 and 0.93, respectively. In potato dough, abundant *S. aureus* growth was found in conjunction with a_w values ranging from 0.88 to 0.97; however, SEB production was only evident at $a_w \geq 0.97$.

It is apparent that modification of a_w can lead to an inhibition of enterotoxin synthesis without a concomitant inhibition of growth (and growth related biochemical reactions). The mechanism underlying this differential response has not been determined, though the observation that different solutes have different effects on enterotoxin synthesis suggests that both physical and physiological effects play a role in the inhibition of enterotoxin biosynthesis. It is possible that by careful selection of humectants used to produce intermediate moisture foods, manufacturers can formulate products that limit the ability of *S. aureus* to produce enterotoxins, thereby reducing the risk of staphylococcal food poisoning.

MINERAL IONS

Only a limited amount of experimentation has been directed toward determining how various cations and anions can influence growth and enterotoxin formation by *S. aureus*. Siderophores, bacterial iron chelating agents, have been detected in *S. aureus* (*67,71*), but their chemical structures and mechanisms have not been identified. Presumably, chelators of other metallic ions are also present in staphylococci.

Morita et al. (*75*) reported that addition of magnesium ions to casein hydrolysate medium stimulated SEB and SEC production but had no effect on SEA synthesis. Addition of Fe^{++} increased SEB synthesis, but did not influence the levels of SEA and SEC. Using a chemically defined medium, Keller et al. (*57*) found that increasing the levels of Mg^{++} (0.4 to 1.5 m*M*), PO_4^{-3} (1.44 to 2.87 m*M*) or K^+ (1.5 to 30 m*M*) resulted in a doubling of SEB synthesis. Addition of other trace metals did not affect SEB production.

Considering that the presence of mineral ions greatly influence both growth of microorganisms and their ability to synthesize extracellular products, it is surprising that there are so few studies examining the effects of mineral content on *S. aureus*. This appears to be an area that warrants further research.

SUBLETHAL INJURY

When *S. aureus* is subjected to sublethal stresses such as heat, cold, freeze-drying, irradiation, or chemicals, it displays evidence of injury (i.e. inability to form colonies on media containing high levels of NaCl) (*15,44*). A major manifestation of injury in *S. aureus* is loss of membrane integrity. Thus cell components such as nucleic acids, amino acids, peptides, membrane lipids and ions can be found in the extracellular environment as responses to in-

jury in *S. aureus* (*42,45,47*). Injured *S. aureus* cells have minimal metabolic capacity (*12*), and there is no synthesis of enterotoxins. However, when injured cells are removed from the stress-producing environment (or the stress is eliminated), they undergo repair, regain salt tolerance, and reinitiate growth. With heat-injured cells, repair occurs when the microorganism is transferred to a medium containing a source of amino acids, glucose, phosphate and magnesium (*42,47*).

Collins-Thompson et al. (*26*) demonstrated that when *S. aureus* was heat-injured and then transferred to microbiological medium, the bacteria repaired injury and subsequently grew and produced SEB. Injured cells underwent a 5- to 6-h lag before growth was initiated, and there was a delay in enterotoxin production as compared to uninjured controls. This lag period presumably represents the time duration needed to accomplish repair. Once the injured cells reinitiated growth, their growth and SEB synthesis were similar to those observed for uninjured cells. Fung and Vandenbosch (*30*) have shown that *S. aureus* cells injured by freeze-drying synthesized SEB when the cells were rehydrated and allowed to repair. Thus the results obtained by Collins-Thompson et al. (*26*) and Fung and Vandenbosch (*30*) suggest that *S. aureus* injured by food processing procedures could potentially repair and initiate toxin production in foods. Recent work in our laboratory with single cell isolates of injured *S. aureus* that have been allowed to recover indicates that the injury process does not affect the microorganism's potential for producing enterotoxin.

There do not appear to have been any documented cases of staphylococcal food poisoning that has involved foods in which injured *S. aureus* have repaired, grown and produced enterotoxin. However, such outbreaks have occurred with other food poisoning microorganisms (e.g., *Salmonella*), and it must be presumed that a similar potential exists for *S. aureus*. If a food is underprocessed, resulting in injury instead of lethality to *S. aureus*, subsequent temperature abuse could produce conditions leading to potential food poisoning problems. The actual significance of sublethal injury for *S. aureus* and other pathogens in foods needs further clarification.

CONCLUDING REMARKS

Past research has demonstrated that growth and enterotoxin production by *S. aureus* are influenced by a variety of environmental and nutritional factors including temperature, pH, a_w, salt levels, inoculum size, competing microbiota, atmospheric composition and carbon and nitrogen sources. However, this work has largely been done in microbiological media where conditions other than the ones being tested are optimized. Whether or not this approach is valid to determine how the microorganism will behave in a food has only been tested to a limited extent. There are suggestions that additional primary and interactive effects need to be characterized in various foods. Adequate characterization of the factors influencing staphylococcal growth and toxin synthesis in foods should then allow a more rational means of modifying formulation and processing parameters to better protect consumers from the potential for staphylococcal food poisoning. Of particular interest is identification of interactions between the various factors affecting the bacteria. This would allow optimization of anti-staphylococcal activity by manipulation of multiple environmental and nutritional parameters. In this manner, the microbiological safety of specific foods can be more accurately predicted, thereby allowing a more rational means of optimizing both safety and production considerations. This coordinated approach to preservation would appear to offer significant advantages, and should be a viable approach if there is an adequate understanding of the factors that influence the growth of foodborne pathogens in foods. Hopefully, future reearch will be directed towards determining the various primary and interactive effects that can be manipulated to control *S. aureus* and other pathogens in foods.

REFERENCES

1. Angelotti, R., M. J. Foter, and K. H. Lewis. 1961. Time-temperature effects on salmonella and staphylococci in foods. I. Behavior in refrigerated foods. Am. J. Public Health 51:76-83.
2. Angelotti, R., M. J. Foter, and K. H. Lewis. 1961. Time-temperature effects on salmonella and staphylococci in foods. II. Behavior at warm holding temperatures. Am. J. Public Health 51:83-88.
3. Archer, D. L. 1978. Immunotoxicology of foodborne substances: an overview. J. Food Prot. 41:983-988.
4. Babel, F. J. 1977. Antibiosis by lactic culture bacteria. J. Dairy Sci. 60:815-821.
5. Baird-Parker, A. C. 1971. Factors affecting the production of bacterial food poisoning toxins. J. Appl. Bacteriol. 34:181-197.
6. Barber, L. E., and R. H. Deibel. 1972. Effect of pH and oxygen tension on staphylococcal growth and enterotoxin formation in fermented sausage. Appl. Microbiol. 24:891-898.
7. Bennett, R. W., and W. T. Amos. 1982. *Staphylococcus aureus* growth and toxin production in nitrogen-packed sandwiches. J. Food Prot. 45:157-161.
8. Bergdoll, M. S. 1973. Enterotoxin detection. pp. 287-292. *In* B. C. Hobbs and J. B. Christian (eds.). The microbiological safety of food. Academic Press, New York, NY.
9. Bergdoll, M. S. 1979. Staphylococcal intoxications. pp. 443-494. *In* H. Riemann and F. L. Bryan (eds.). Food-borne infections and intoxications, second ed., Academic Press, New York, NY.
10. Bergdoll, M. S., and R. N. Robbins. 1973. Characterization of types of staphylococcal enterotoxins. J. Milk Food Technol. 36:610-612.
11. Bergdoll, M. S., R. F. Reiser, B. A. Crass, R. N. Robbins, and J. P. Davis. A new staphylococcal enterotoxin, enterotoxin F, associated with toxic-shock-syndrome *Staphylococcus aureus* isolates. The Lancet, May 9, 1981, pp. 1017-1021.
12. Bluhm, L., and Z. J. Ordal. 1969. Effect of sublethal heat on the metabolic activity of *Staphylococcus aureus*. J. Bacteriol. 97:140-150.
13. Bryan, F. L. 1976. *Staphylococcus aureus*. pp. 12-128. *In* M. P. Defigueiredo and D. F. Splittstoesser (eds.). Food microbiology: public health and spoilage aspects. AVI Publishing Co., Inc., Westport, CT.
14. Buchanan, R. L., and Solberg, M. 1972. Interaction of sodium nitrate, oxygen and pH on growth of *Staphylococcus aureus*. J. Food Sci. 37:81-85.
15. Busta, F. F. 1976. Practical implications of injured microorganisms in foods. J. Milk Food Technol. 39:138-145.
16. Carpenter, D. F., and G. J. Silverman. 1974. Staphylococcal enterotoxin B and nuclease production under controlled dissolved oxygen conditions. Appl. Microbiol. 28:628-637.

17. Carpenter, D. F., and G. J. Silverman. 1976. Synthesis of staphylococcal enterotoxin A and nuclease under controlled fermentor conditions. Appl. Environ. Microbiol. 31:243-248.
18. Casman, E. P., D. W. McCoy, and P. J. Brandly. 1963. Staphylococcal growth and enterotoxin production in meat. Appl. Microbiol. 11:498-500.
19. Centers for Disease Control. 1976. Foodborne and waterborne disease outbreaks annual summary 1975, issued Sept. 1976.
20. Centers for Disease Control. 1977. Foodborne and waterborne disease outbreaks annual summary 1976, issued Oct. 1977.
21. Centers for Disease Control. 1979. Foodborne and waterborne disease outbreaks annual summary 1977, issued Aug. 1979.
22. Centers for Disease Control. 1981. Foodborne disease outbreaks annual summary 1978 (revised), reissued Feb. 1981.
23. Centers for Disease Control. 1981. Foodborne disease outbreaks annual summary 1979, issued April 1981.
24. Chordash, R. A., and N. N. Potter. 1976. Stability of staphylococcal enterotoxin A to selected conditions encountered in foods. J. Food Sci. 41:906-909.
25. Collins-Thompson, D. L., B. Aris, and A. Hurst. 1973. Growth and enterotoxin B Synthesis by *Staphylococcus aureus* S-6 in associative growth with *Pseudomonas aeruginosa*. Can. J. Microbiol. 19:1197-1201.
26. Collins-Thompson, D. L., A. Hurst, and H. Kruse. 1973. Synthesis of enterotoxin B by *Staphylococcus aureus* strain S-6 after recovery from heat injury. Can. J. Microbiol. 19:1463-1468.
27. Dietrich, G. G., R. J. Watson, and G. J. Silverman. 1972. Effect of shaking speed on the secretion of enterotoxin B by *Staphylococcus aureus*. Appl. Microbiol. 24:561-566.
28. DiGiacinto, J. V., and W. C. Frazier. 1966. Effect of coliform and *Proteus* bacteria on growth of *Staphylococcus aureus*. Appl. Microbiol. 14:124-129.
29. Donnelly, C. B., J. E. Leslie, and L. A. Black. 1968. Production of enterotoxin A in milk. Appl. Microbiol. 16:917-924.
29a. Finne, G. 1982. Modified- and controlled-atmosphere storage of muscle foods. Food Technol. 36(2):128-133.
30. Fung, D. Y. C., and L. L. Vandenbosch. 1975. Repair, growth, and enterotoxigenesis of *Staphylococcus aureus* S-6 injured by freeze-drying. J. Milk Food Technol. 38:212-218.
31. Genigeorgis, C. A. 1976. Quality control for fermented meats. J. Am. Vet. Med. Assoc. 169:1220-1228.
32. Genigeorgis, C., and W. W. Sadler. 1966. Effect of sodium chloride and pH on enterotoxin B production. J. Bacteriol. 92:1383-1387.
33. Genigeorgis, C., H. Riemann, and W. W. Sadler. 1969. Production of enterotoxin B in cured meats. J. Food Sci. 34:62-68.
34. Genigeorgis, C., M. Savoukidis, and S. Martin. 1971. Initiation of staphylococcal growth in processed meat environments. Appl. Microbiol. 21:940-942.
35. Genigeorgis, C., M. S. Foda, A. Mantis, and W. W. Sadler. 1971. Effect of sodium chloride and pH on enterotoxin C production. Appl. Microbiol. 21:862-866.
36. Goepfert, J. M., and H. U. Kim. 1975. Behavior of selected foodborne pathogens in raw ground beef. J. Milk Food Technol. 38:449-452.
37. Graves, R. R., and W. C. Frazier. 1963. Food microorganisms influencing the growth of *Staphylococcus aureus*. Appl. Microbiol. 11:513-516.
38. Haines, W. C., and L. G. Harmon. 1973. Effect of variations in conditions of incubation upon inhibition of *Staphylococcus aureus* by *Pediococcus cerevisiae* and *Streptococcus lactis*. Appl. Microbiol. 25:169-172.
39. Harvey, J., J. T. Patterson, and P. A. Gibbs. 1982. Enterotoxigenicity of *Staphylococcus aureus* strains isolated from poultry: raw poultry carcases as a potential food-poisoning hazard. J. Appl. Bacteriol. 52:251-258.
40. Hojvat, S. A., and H. Jackson. 1969. Effects of sodium chloride and temperature on the growth and production of enterotoxin B by *Staphylococcus aureus*. Can. Inst. Food Technol. J. 2:56-59.
41. Holt, J. G. (ed.) 1977. The shorter Bergey's manual of determinative bacteriology, 8th ed. Williams and Wilkins Co., Baltimore, MD.
42. Hughes, A., and A. Hurst. 1976. Magnesium requirements of *Staphylococcus aureus* for repair from sublethal injury. Can. J. Microbiol. 22:1202-1205.
43. Hughes, A., and A. Hurst. 1980. The effect of NaCl on the upper temperature limit for growth of and enterotoxin synthesis by *Staphylococcus aureus*. Can. J. Microbiol. 26:507-510.
44. Hurst, A. 1977. Bacterial injury: a review. Can. J. Microbiol. 23:935-944.
45. Hurst, A., A. Hughes, J. L. Beare-Rogers, and D. L. Collins-Thompson. 1973. Physiological studies on the recovery of salt tolerance by *Staphylococcus aureus* after sublethal heating. J. Bacteriol. 116:901-907.
46. Hurst, A., A. Hughes, and R. Pontefract. 1980. Mechanism of the temperature protective effect of salts on *Staphylococcus aureus*. Can. J. Microbiol. 26:511-517.
47. Iandolo, J. J., and Z. J. Ordal. 1966. Repair of thermal injury of *Staphylococcus aureus*. J. Bacteriol. 91:134-142.
48. Iandolo, J. J., and W. M. Shafer. 1977. Regulation of staphylococcal enterotoxin B. Infect. Immun. 16:610-616.
49. Ibrahim, G. F., D. R. Radford, A. K. Baldock, and L. B. Ireland. 1981. Inhibition of growth of *Staphylococcus aureus* and enterotoxin production in cheddar cheese produced with induced starter failure. J. Food Prot.44:189-193.
50. Ikram, M., and L. O. Luedecke. 1977. Growth and enterotoxin A production by *Staphylococcus aureus* in fluid dairy products. J. Food Prot. 40:769-771.
51. Jarvis, A. W., R. C. Lawrence, and G. G. Pritchard. 1973. Production of staphylococcal enterotoxins A, B, and C under conditions of controlled pH and aeration. Infect. Immun. 7:847-854.
52. Jarvis, A. W., R. C. Lawrence, and G. G. Pritchard. 1975. Glucose repression of enterotoxins A, B, and C and other extracellular proteins in staphylococci in batch and continuous culture. J. Gen. Microbiol. 86:75-87.
53. Johnson, H. M., G. J. Stanton, and S. Baron. 1977. Relative ability of mitogens to stimulate production of interferon by lymphocyte cells and to induce suppression of the in vitro immune response. Proc. Soc. Exp. Biol. Med. 154:138-141.
54. Józefczyk, Z. 1974. Specific human antibodies to enterotoxins A, B, and C_1 of *Staphylococcus:* their increased synthesis in staphylococcal infections. J. Inf. Diseases 130:1-7.
55. Kao, C. T., and W. C. Frazier. 1966. Effect of lactic acid bacteria on growth of *Staphylococcus aureus*. Appl. Microbiol. 14:251-255.
56. Kato, E., M. Khan, L. Kujovich, and M. S. Bergdoll. 1966. Production of enterotoxin A. Appl. Microbiol. 14:966-972.
57. Keller, G. M., R. S. Hanson, and M. S. Bergdoll. 1978. Effect of minerals on staphylococcal enterotoxin B production. Infect. Immun. 20:158-160.
58. Keller, G. M., R. S. Hanson, and M. S. Bergdoll. 1978. Molar growth yields and enterotoxin B production of *Staphylococcus aureus* S-6 with amino acids as energy sources. Infect. Immun. 20:151-157.
59. Langford, M. P., G. J. Stanton, and H. M. Johnson. 1978. Biological effects of staphylococcal enterotoxin A on human peripheral lymphocytes. Infect. Immun. 22:62-68.
60. Lee, I. C., K. E. Stevenson, and L. G. Harmon. 1977. Effect of beef broth protein on the thermal inactivation of staphylococcal enterotoxin B. Appl. Environ. Microbiol. 33:341-344.
61. Lee, I. C., L. G. Harmon, and J. F. Price. 1977. Growth and enterotoxin production by staphylococcal in genoa salami. J. Food Prot.40:325-329.
62. Lee, R. Y., G. J. Silverman, and S. T. Munsey. 1981. Growth and enterotoxin A production by *Staphylococcus aureus* in precooked bacon in the intermediate moisture range. J. Food Sci. 46:1687-1700.
63. Lee, W. H., C. L. Staples, and J. C. Olson, Jr. 1975. *Staphylococcus aureus* growth and survival in macaroni dough and the persistence of enterotoxins in the dried products. J. Food Sci. 40:119-120.

64. Lotter, L. P., and L. Leistner. 1978. Minimal water activity for enterotoxin A production and growth of *Staphylococcus aureus*. Appl. Environ. Microbiol. 36:377-380.
65. McCoy, D. W., and J. E. Faber. 1966. Influence of food microorganisms on staphylococcal growth and enterotoxin production in meat. Appl. Microbiol. 14:372-377.
66. McLean, R. A., H. D. Lilly, and J. A. Alford. 1968. Effects of meat-curing salts and temperature on production of staphylococcus enterotoxin B. J. Bacteriol. 95:1207-1211.
67. Marcelis, J. H., H. J. den Daas-Slagt, and J. A. A. Hoogkamp-Korstanje. 1978. Iron requirement and chelator production of staphylococci, *Streptococcus faecalis* and enterobacteriaceae. Antonie van Leeuwenhoek 44:257-267.
68. Markus, Z., and G. J. Silvermann. 1968. Enterotoxin B production by nongrowing cells of *Staphylococcus aureus*. J. Bacteriol. 96:1446-1447.
69. Markus, Z., and G. J. Silverman. 1969. Enterotoxin B synthesis in replicating and nonreplicating cells of *Staphylococcus aureus*. J. Bacteriol. 97:506-512.
70. Markus, Z. H., and G. J. Silverman. 1970. Factors affecting the secretion of staphylococcal enterotoxin A. Appl. Microbiol. 20:492-496.
71. Maskell, J. P. 1980. The functional interchangeability of enterobacterial and staphylococcal iron chelators. Antonie van Leeuwenhoek 46:343-351.
72. Metzger, J. F., A. D. Johnson, W. S. Collins II, and V. McGann. 1973. *Staphylococcus aureus* enterotoxin B release (excretion) under controlled conditions of fermentation. Appl. Microbiol. 25:770-773.
73. Minor, T. E., and E. H. Marth. 1972. Loss of viability by *Staphylococcus aureus* in acidified media. Inactivation by several acids, mixtures of acids, and salts of acids. J. Milk Food Technol. 35:191-196.
74. Minor, T. E., and E. H. Marth. 1976. Staphylococci and their significance in foods. Elsevier Scientific Publishing Co., New York, NY.
75. Morita, T. N., J. E. Patterson, and M. J. Woodburn. 1979. Magnesium and iron addition to casein hydrolysate medium for production of staphylococcal enterotoxins A, B, and C. Appl. Environ. Microbiol. 38:39-42.
76. Morse, S. A., and J. N. Baldwin. 1971. Regulation of staphylococcal enterotoxin B: effect of thiamine starvation. Appl. Microbiol. 22:242-249.
77. Morse, S. A., and R. A. Mah. 1973. Regulation of staphylococcal enterotoxin B: effect of anaerobic shock. Appl. Microbiol. 25:553-557.
78. Morse, S. A., R. A. Mah, and W. J. Doubrogosz. 1969. Regulation of staphylococcal enterotoxin B. J. Bacteriol. 98:4-9.
79. Noleto, A. L., and M. S. Bergdoll. 1980. Staphylococcal enterotoxin production in the presence of nonenterotoxigenic staphylococci. Appl. Environ. Microbiol. 39:1167-1171.
80. Olson, D. G., and R. N. Terrell. 1981. Sensory properties of processed meats using various sodium salt substitutes. Proc. Meat Indust. Conf., March 26-27, 1981, Chicago, IL. Issued by Am. Meat Institute Found., Arlington, VA.
81. Payne, D. N., and J. M. Wood. 1974. The incidence of enterotoxin production in strains of *Staphylococcus aureus* isolated from foods. J. Appl. Bacteriol. 37:319-325.
82. Perlman, R. L., B. de Crombrugghe, and I. Pastan. 1969. Cyclic AMP regulates catabolite and transient repression in *E. coli*. Nature (London) 223:810-812.
83. Reali, D. 1982. Enterotoxin A and B production in strains of *Staphylococcus aureus* isolated from human beings and foods. J. Hyg. Camb. 88:103-106.
84. Reichert, C. A., and D. Y. C. Fung. 1976. Thermal inactivation and subsequent reactivation of staphylococcal enterotoxin B in selected liquid foods. J. Milk Food Technol. 39:516-520.
85. Reiser, R. F., and K. F. Weiss. 1969. Production of staphylococcal enterotoxins A, B, and C in various media. Appl. Microbiol. 18:1041-1043.
86. Scheusner, D. L., and L. G. Harmon. 1973. Growth and enterotoxin production by various strains of *Staphylococcus aureus* in selected foods. J. Food Sci. 38:474-476.
87. Scheusner, D. L., L. L. Hood, and L. G. Harmon. 1973. Effect of temperature and pH on growth and enterotoxin production by *Staphylococcus aureus*. J. Milk Food Technol. 36:249-252.
88. Seminiano, E. N., and W. C. Frazier. 1966. Effect of pseudomonads and *Archromobacteraceae* on growth of *Staphylococcus aureus*. J. Milk Food Technol. 29:161-164.
89. Smith, B. G., and H. M. Johnson. 1975. The effect of staphylococcal enterotoxins on the primary in vitro immune response. J. Immunol. 115:575-578.
90. Smith, J. L., and S. A. Palumbo. 1980. Inhibition of aerobic and anaerobic growth of *Staphylococcus aureus* in a model sausage system. J. Food Safety 4:221-233.
91. Tatini, S. R. 1973. Influence of food environments on growth of *Staphylococcus aureus* and production of various enterotoxins. J. Milk Food Technol. 36:559-563.
92. Tatini, S. R. 1976. Thermal stability of enterotoxins in food. J. Milk Food Technol. 39:432-438.
93. Tatini, S. R., J. J. Jezeski, J. C. Olson, Jr., and E. P. Casman. 1971. Factors influencing the production of staphylococcal enterotoxin A in milk. J. Dairy Sci. 54:312-320.
94. Tatini, S. R., W. D. Wesala, J. J. Jezeski, and J. A. Morris. 1973. Production of staphylococcal enterotoxin A in blue, brick, mozzarella, and Swiss cheeses. J. Dairy Sci. 56:429-435.
95. Thatcher, F. S., J. Robinson, and I. Erdman. 1962. The 'vacuum pack' method of packaging foods in relation to the formation of the botulinum and staphylococcal toxins. J. Appl. Bacteriol. 25:120-124.
96. Todd, E., R. Szabo, H. Robern, T. Gleeson, C. Park, and D. S. Clark. 1981. Variation in counts, enterotoxin levels and TNase in Swiss-type cheese contaminated with *Staphylococcus aureus*. J. Food Prot. 41:839-848.
97. Troller, J. A. 1971. Effect of water activity on enterotoxin B production and growth of *Staphylococcus aureus*. Appl. Microbiol. 21:435-439.
98. Troller, J. A. 1973. Effect of water activity and pH on staphylococcal enterotoxin B production. Acta Aliment. 2:351-360.
99. Troller, J. A., and J. V. Stinson. 1975. Influence of water activity on growth and enterotoxin formation by *Staphylococcus aureus* in foods. J. Food Sci. 40:802-804.
100. Troller, J. A., and J. V. Stinson. 1978. Influence of water activity on the production of extracellular enzymes by *Staphylococcus aureus*. Appl. Environ. Microbiol. 35:521-526.
101. Vandenbosch, L. L., D. Y. C. Fung, and M. Widomski. 1973. Optimum temperature for enterotoxin production by *Staphylococcus aureus* S-6 and 137 in liquid medium. Appl. Microbiol. 25:498-500.
102. Wieneke, A. A. 1974. Enterotoxin production by strains of *Staphylococcus aureus* isolated from foods and human beings. J. Hyg., Camb. 73:255-262.
103. Wolfe, S. K. 1980. Use of CO- and CO_2-enriched atmospheres for meat, fish, and produce. Food Technol. 34(3):55-58; 63.
104. Woodburn, M., T. N. Morita, and S. Z. Venn. 1973. Production of staphylococcal enterotoxins A, B, and C in colloidal dispersions. Appl. Microbiol. 25:825-833.
105. Zehren, V. L., and V. F. Zehren. 1968. Relation of acid development during cheesemaking to development of staphylococcal enterotoxin A. J. Dairy Sci. 51:645-649.